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What is hidden in the pannexin treasure trove: the sneak peek and the guesswork

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Abstract

Connexins had been considered to be the only class of the vertebrate proteins capable of gap junction formation; however, new candidates for this function with no homology to connexins, termed pannexins were discovered. So far three pannexins were described in rodent and human genomes: Panx1, Panx2 and Panx3. Expressions of pannexins can be detected in numerous brain structures, and now found both in neuronal and glial cells. Hypothetical roles of pannexins in the nervous system include participating in sensory processing, hippocampal plasticity, synchronization between hippocampus and cortex, and propagation of the calcium waves supported by glial cells, which help maintain and modulate neuronal metabolism. Pannexin also may participate in pathological reactions of the neural cells, including their damage after ischemia and subsequent cell death. Recent study revealed non-gap junction function of Panx1

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hemichannels in erythrocytes, where they serve as the conduits for the ATP release in response to the osmotic stress. High-throughput studies produced some evidences of the pannexin involvement in the process of tumorigenesis. According to brain cancer gene expression database REMBRANDT, PANX2 expression levels can predict post diagnosis survival for patients with glial tumors. Further investigations are needed to verify or reject hypotheses listed.

Keywords: gap junction • hemichannel • pannexin • synchronous activity • calcium waves • hippocampus • glioma • ATP release

Introduction

In the majority of the organized tissues cells are connected by various types of intercellular junctions, among which gap junctions are prominent players. Gap junctions (GJs) are essential for functional coordination and homeostasis within tissues, as they permit the direct cell-to-cell exchange of small molecules (up to 1 kDa in molecular mass). In fact, every GJ is the channel formed by pairs of hemichannels opposing the narrow intercellular gap. Under certain conditions non-junctional hemichannels may be also formed; these channels are open to the extracellular space [1, 2]. The gating, conductivity, and selectivity of GJ depends on the protein composition of the channel, the number of the channels in the membrane, transjunctional gradients, and other factors [3, 4].

Connexins (Cx) had been considered to be the only class of the vertebrate proteins capable of GJ formation, however, new candidates for this function with no homology to connexins were discovered. The term pannexins was coined, underscoring the presence of these family homologues in all groups of multicellular animals [5, 6]. The identification of mammalian pannexins and the prediction of their function were based on initial invertebrate model studies [7–11]. Subsequently, the ability to form intercellular channels in paired oocytes has been proven for rodent pannexins [12, 13].

Gap junction forming proteins: evolutionary considerations

Despite vast literature describing various properties of GJs in multicellular organisms, the general picture is far from clear. One hope for improve in the understanding of GJ function may come from an evolutionary approach. The evolutionary aspects of GJ proteins were recently reviewed [14] and can be briefly summarized as follows. GJs appear to be universal feature of Metazoans. Connexins were the first identified family of GJ proteins. It now appears that connexins are specific only to Chordates (vertebrates and tunicates). Numerous attempts to find connexins in invertebrates have failed, so alternatives to connexin GJ protein candidates were investigated. Finally, it was suggested that invertebrate GJs are assembled from proteins unrelated to the connexin gene family, innexins [7–11]. Although connexins and innexins have very different primary sequences they nonetheless have some similar features. Proteins of both unrelated families have similar topology with four transmembrane domains.

Recently, genes encoding innexin homologues were discovered in different taxonomic groups, including Chordates [5, 6, 12; 15, 16, 111]. We proposed re-classification of innexins and their vertebrate homologues into a larger family termed pannexins (from the Greek “pan” – “all, throughout” and Latin “nexus” - “connection, bond”). Some authors call innexins and their vertebrate homologues a “superfamily” [112]; others authors question the homology between pannexins in vertebrate and invertebrate innexins [17] or discuss the degree of such homology [111]. As pannexin profile easily identifies both chordate and non chordate pannexins (*e.g.* with query by PROSITE: PS51013, www.expasy.org/prosite/profile), in our view, there is obvious sequence similarity between vertebrate pannexins and their invertebrate counterparts. Pannexins seem to be ubiquitous metazoan proteins, whereas connexins appear to be chordate specific. The growing body of recent sequence data from different organisms largely supports these conclusions. Indeed, no connexins were found among animals outside Chordata and more innexins were discovered in various Metazoans [16, 18]. However, some complications to this simple description started to emerge. In several metazoans genomes sufficiently covered by raw sequencing runs neither connexins nor pannexins could be identified. For instance, the

search by TBLASTN or TFASTX programs for putative pannexins and connexins in *Nematostella vectensis* (starlet sea anemone, Cnidaria, Anthozoa) and *Strongylocentrotus purpuratus* (purple sea urchin, Echinodermata) genomes revealed no reliable hits. In sequence trace files of sponge *Reniera*, only a single entry corresponding to an apparent pannexin could be found. Unfortunately, this entry seems questionable, as it matches closely to molluscan pannexins and did not match any other *Reniera* sequences. It, therefore, most likely represents a contamination artifact. This opens intriguing possibility that genomes of some multicellular organisms do not contain any genes encoding members of known GJ protein families. At the same time physiological data supporting the existence of GJs are convincing for sea urchin [19] and Anthozoa [20]; such that the postulate regarding ubiquity of the GJs in Metazoa should hold true. Although, we ourselves named pannexins, considering them as universal metazoan GJ protein as opposed to Chordate-specific connexins, now it is necessary to admit the fact that we are facing a possibility of the discovery of additional protein families utilized for GJ function. If such hypothetical N-nexins would be found in sea urchins, sponges, or other animals, the possibility that they also exist in humans and other mammals is thrilling.

Functional properties of pannexin channels and hemichannels

Formation of the channels and the gating characteristics

So far three pannexin encoding genes were described in rodent and human genomes: Panx1, Panx2 and Panx3 [12, 6]. Panx1 is expressed in numerous organs in mouse, rat and human, including both developing and mature nervous systems. The expression of the Panx2 gene is found in many organs in rodents, but restricted to nervous system in human, while expression of Panx3 confirmed so far only in rat's skin (see Table 1). *In silico* search revealed presence of Panx3 in few more structures and tissues, particularly in the cartilage (Table1).

The gating properties of pannexin channels were studied by Bruzzone *et al.* (2003) using a heterologous expression system in *Xenopus* oocytes [12]. Substantial voltage-activated currents were consistently induced when single oocytes expressing Panx1 were stimulated by voltage steps >-20 mV. In contrast, neither Panx2 nor Panx3 induced membrane currents under the same voltage step conditions while expressed alone [12]. These findings suggest that Panx2 and Panx3 are not able to form functional homomeric hemichannels, at least in oocytes. Expanding upon results of the experiments performed in the single oocytes, Panx1 was found to function as a channel both alone and in combination with Panx2, whereas Panx2 alone was not able to form a channel [12].

Homomeric and heteromeric hemichannels formed by pannexins can differ in their gating properties [12]. Panx1 and Panx2 are able to form heteromeric hemichannels; however, currents recorded from oocytes coexpressing Panx1 with Panx2 are smaller than currents measured from cells injected with the same amount of Panx1 mRNA. Imposition of a voltage step leads to a significant delay in achieving a peak current by Panx1/Panx2 coexpressing cells compared to Panx1-expressing cells [12]. This phenomenon might be explained by slower opening or closing of certain pannexin hemichannels (or both).

Recently, the same team of researchers published additional data on pharmacological properties of pannexin hemichannels [13], in particular, their remarkable sensitivity to blockade by carbenoxolone (CBX), a reversible inhibitor reducing connexin channel conductance. Threshold concentration of CBX needed for inhibition of pannexins hemichannels is lower then for connexin46 (Cx46) hemichannels (Cx46 has been extensively studied as the prototype of the hemichannel forming connexins). At the same time, the magnitude of the CBX effect is even higher for pannexins than for Cx46. In addition, connexin inhibiting flufenamic acid suppresses pannexin hemichannels only modestly, thus indicating that this GJ blocker affects connexins and pannexins in the different ways [13].

Possible functions of the pannexins

So far, hypotheses describing pannexin functions in the mammalian brain center around two scenarios. One focuses on the suggestion that intercellular pannexin channels might represent a novel class of elec-

Table 1 Expression of pannexins in rodents and human.

Pannexin	Mouse	Rat	Human
Panx1	CNS (embryonic stages E13.5, E15–E18). [b] Lung++, spleen++, heart+, testis+, kidney+, spinal cord++, brain++ (cortex++, cerebellum++, brain stem+/-, olfactory bulb++, retina++) [c]	Adrenal gland+, bladder+, eye+, spinal cord+, thyroid+, prostate+, stomach+, large intestine+, liver+, kidney+/-, brain+ (cortex+, striatum+, olfactory bulb++, hippocampus+, thalamus+, cerebellum++) [a] CNS (spinal cord+, cerebellum++, cortex+, hippocampus+, olfactory bulb++) [d]	Heart++, skeletal muscle++, testis++, ovary++, brain+, placenta+, kidney+, spleen+, thymus+, prostate+, small intestine+, lung+/-, liver+/-, pancreas+/-, spleen+/-, colon+/-, peripheral blood leukocytes+/- [b] Erythrocytes+, endothelial cells+ [e] Platelets+ [f]
Panx2	CNS (cerebellum+, hippocampus+, mamillary nuclei+, substance nigra+, cortex+, amygdala+) [b], CNS (olfactory bulb++, hippocampus+, neocortex+, basal telencephalon+, thalamus+, hypothalamus+, midbrain+, hindbrain+, cerebellum+) this paper	spinal cord++, thyroid+, prostate+, liver+, kidney+, eye++, brain++ (olfactory bulb++, hippocampus++, cortex++, cerebellum++, thalamus+, striatum+) [a] CNS (spinal cord+, cerebellum++, cortex+, hippocampus+, olfactory bulb++) [d]	CNS (cerebellum++, cerebral cortex++, medulla++, occipital lobe++, frontal lobe++, temporal lobe++, putamen+), but not spinal cord. Not found in any non-neuronal tissue tested (represented by Clontech MTN blots) [b]
Panx3 (experimental evidences)		Skin [a]	Not found in any tissues from adult human organs tested (represented by Clontech MTN blots) [b]
Panx3 (*in silico* search, according to UniGene)	Embryo, osteoblasts++, synovial fibroblasts++, whole joints, inner ear, skin, vagina	Cartilage, cartilage tumors, osteoblasts	Embryo

++ abundant expression; + intermediate expression; +/- low to undetectable expression

References:

[a] - Bruzzone *et al.*, 2003
[b] - Baranova *et al.*, 2004
[c] - Ray *et al.*, 2005
[d] - Vogt *et al.*, 2005
[e] - Locovei *et al.*, 2006
[f] - Moebius *et al.*, 2005

trical synapses that, in particular, may play a role in the generation of oscillatory and synchronous activity in number of brain structures (see chapter III for details). Another hypothesis suggests that pannexin hemichannels may be involved in extracellular release of small molecules and, particularly, may promote the propagation of the calcium waves.

The second hypothesis implying pannexins mediating propulsion of the long-range calcium signals recently received several considerable corrobo-

rations. In the nervous system, calcium waves can spread through glial cells to neurons thus representing a system of widespread non-synaptic communication [23]. Neurons can signal back to glial cells through multiple pathways. Once initiated, synaptic and non-synaptic activities of neurons trigger an increase in astrocytic intracellular calcium concentration. Localized signaling responses in individual astrocytes can then be widely propagated in the form of intercellular calcium waves, which can be visualized with fluorescent Ca^{2+} indicators. There are two main mechanisms of calcium wave propagation suggested: one is dependent on GJs between cells, and is well established [24], and another is based on the release of extracellular signaling molecules, particularly adenosine triphosphate (ATP), that triggers subsequent ATP-induced activation of purinergic membrane receptors on the neighboring cells. In turn, activation of purinergic receptors may lead to release of ATP from adjacent cells, thus endorsing calcium wave propagation. In that way, long-range intercellular calcium signaling in astrocytes allows spatial coordination of their function as the supporters of the neuronal metabolism [23].

Potential mechanism suggested for the ATP release is implying an existence of large-pore hemichannels. Panx1 is an attractive candidate for this role, as it is able to form functional and efficient hemichannels at physiologic calcium concentrations [12] and is expressed in many organs and tissues capable of calcium wave propagations. As opposed to connexins, pannexin channels are able to open when external concentration of calcium ions is at a physiological level, thus their permeability in normal tissues is ensured [13]. Patch clamp experiments reveal that pannexin hemichannels are voltage-dependent and are activated in the response to the mechanical stress [21]. Under conditions favoring channel opening, pannexin hemichannels elevate ATP efflux [21]. Recently, another study from the same lab [22] demonstrated that pannexin channels are also involved in ATP-induced ATP release, the process typical for calcium wave propagation. According to the current model, such release may be triggered by extracellular ATP through purinergic receptors [25]. Locovei *et al.* (2006) co-expressed pannexin1 in *Xenopus* oocytes with the purinergic receptors P2Y1 or P2Y2 [22]. Application of ATP to oocytes co-expressing Panx1 together with either of two P2Y receptors was sufficient to activate pannexin hemichannels, and led to large currents. It is interesting to note that P2Y2-dependent activation was fast and transient while P2Y1 activated the channels slowly and produced sustained opening of the channel [22]. These findings remarkably parallel the observation that P2Y2 receptors promote propagation of the calcium waves at higher velocity than P2Y1 receptors [26].

If pannexins are playing role in calcium wave propagation, corresponding hemichannels should be activated by an increase in the concentration of intracellular calcium. This statement has been supported by the data collected after patch clamp experiments performed with excised inside-out membrane patches [22]. In these experiments application of micromolar concentrations of calcium to the cytoplasmic face of the channels resulted in channel activity that ceased upon washout of the calcium containing solution, suggesting active pannexin involvement in calcium permeability changes. Locovei *et al.*, 2006 proposed ATP-dependent model of calcium wave propagation which implies that P2Y receptors activate phospholipase C. This event, in turn, produces IP3 capable of calcium release from its stores and an increase in its intracellular concentration, with subsequent release of new portion of ATP, and thus further propagates the wave. Taken together, these data suggest that properties of the Panx1 hemichannels revealed by described above experiments are very similar to properties of the hypothetical hemichannels responsible for calcium wave propagation. Nevertheless, further experiments utilizing pannexin inhibitors or siRNA suppression of Panx1 hemichannel are necessary.

The results of the recently published study of the Panx1 function in human erythrocytes are even more intriguing [113]. Erythrocytes do not express Cx43 that has been implicated in ATP release by some other authors [113, 114]. Moreover, there is no evidence that erythrocytes form intercellular GJ channels. Locovei and co-authors found that not only these cells express Panx1 and form hemichannels, but that the ATP efflux from erythrocytes is affected by CBX [113]. As we already mentioned, pannexins exhibit a remarkable sensitivity to blockade by CBX, exceeding that of the connexin [13]. Also, the gating properties of the ATP channels in erythrocytic membranes are consistent with these of pannexin hemichannels [113]. Taking together, these findings indicate that the formation of the transient hemichannels connecting

the cytoplasm with extracellular space may be an indispensable function of the pannexins.

In addition to the cytoplasm to extracellular space connections and to the role in the electrical synapses, pannexins may participate in a neuronal death after ischemia. Both pre- and post-treatments with CBX prevent stroke injury after the ischemic insult in the rat hippocampus, resulting in decreased numbers of TUNEL-positive neurons [27]. On the other hand, treatment with quinine, an inhibitor of Cx36-mediated gap junctional coupling, was unable to limit the spread of ischemia [27]. Therefore, it was hypothesized that GJ molecules other than connexins are central for the ischemia, and pannexins might be among them. This hypothesizes was further supported by recently found similarity of the biophysical properties of the hemichannels opened in acutely isolated hippocampal neurons under ischemic conditions and homomeric Panx1 hemichannels expressed in oocytes [115]. It was suggested that the opening of Panx1 hemichannels could mediate dysregulated ionic fluxes, anoxic depolarization as well as energy depleting efflux of glucose and ATP characteristic for ischemic neurons [115]. It is tempting to speculate that pannexin may represent a new class of pharmacological targets in the stroke neurology.

Beside that, in mouse heart Panx1 expression significantly increases as result of the selective ERK, p38 and JNK activations [116]. These findings were obtained in the high-throughput study of adult mice expressing activated mutants of Ras, MKK3 and MKK7 in the cardiac specific and temporally regulated fashion. Panx1 was one of 36 genes which expression was significantly altered in all three transgenic models. It is of interest that all three branches of MAP kinase signaling contribute to various cardiomyopathies. Molecular events accompanying cardiac failures are intimately interwoven with events involved in the cardiac ischemia and the cardiac cell death [117]. Since pannexins were shown to be involved in the ischemic death of the neurons [115], it is important to study pannexin connection to ischemia in the heart.

Possible ways to regulate pannexin channels

Multiple regulatory partners have been described for the connexins, major family of GJ proteins in mammals. As pannexins are suggested to participate in various GJ and non-GJ processes, one may inquire about their possible regulators. Indeed, if these channels are to be kept open, cell constituents would rapidly leak out of the cell, causing cell death. Therefore, deactivation mechanisms should exist to provide gating control for potentially harmful pannexin hemichannel. Although mechanism for negative feedback has not been yet proposed, at least two possible explanations exist for pannexin regulation. First possibility concerns pannexin channel activation by regulatory kinases. A number of conservative serine and threonine phosphorylation sites were predicted both in the middle cytoplasmic loops and carboxyl-terminal tails of pannexins [17]. These sites are especially abundant in Panx2 that have more restricted expression pattern in comparison to the ubiquitously expressed Panx1. The regulatory kinases predicted to modify pannexins include such important enzymes as PKC, PKA, PKG and CKII [17]. In addition, a homology search in the Swissprot sequence database reveal that Panx2 contains IKK phosphorylation motif, indicating probable intersection of pannexin signaling with NF-kB pro-inflammatory cascade [28]. The most elusive member of pannexin family, Panx3, contains a tyrosine phosphorylation consensus site for the EGFR (Epidermal Growth Factor Receptor) [17]. Therefore, it may not be coincidental that EGFR is expressed in chondrocytes, one of the few cell types that, according to UniGene, synthesize Panx3 (see Table 1). Since EGFR is able to promote chondrocyte proliferation and stimulate proton efflux [29], one might speculate that Panx3 might participate in these processes. Another source of information pertinent to the regulation of pannexins is the experiments performed in yeast two-hybrid systems. Thus far, such experiments yielded a regulatory β-subunit of voltage-dependent potassium channel ($K_v\beta3$ protein) as a confirmed partner of Panx1 [30]. Association of β-subunits with α-subunits of the voltage gated potassium channel confers its rapid inactivation by a ball domain in the Kv-β amino terminus [31]. As the velocity of the inactivation is dependent on the intracellular redox potential [32], and as the calcium wave amplitude, velocity and interwave periods are strengthened by oxidizable substrates that energize mitochondria [33], it is tempting to speculate that $K_v\beta3$ interaction with Panx1 is an integral part of the wave propagation process.

Pannexin gene expression in the brain of the rodents

Pannexin gene expression in the eyes of the rodents

Both Panx1 and Panx2 are synthesized in the eye of the rat, the signal for Panx2 was found to be especially abundant [12]. Detailed studies of Panx1 mRNA distribution in the adult mouse retina revealed a prominent staining for Panx1 mRNA in the ganglion cell layer (GCL), the inner nuclear layer (INL) and in the outermost cells of the outer nuclear layer (ONL). In addition, few scattered positive cells were observed in the inner plexiform layer (IPL) [34]. These findings were confirmed by a laser capture microdissection combined with RT-PCR [34, 35].

Another recent study described robust expression of both Panx1 and Panx2 in mouse and rat retina, with predominant accumulation in the GCL and at some extent in the INL of adult rodents. Fine grains of punctuate labeling were present throughout all other layers [36]. Staining with anti-Panx1 antibodies highlighted retinal ganglion cells, their processes and also amacrine and horizontal cells in newborn and young mice (P20). Morphology of the stained cells indicated that in GCL expression of pannexins is attributed to the ganglion cells, but not to the satellite glial cells. Retrograde co-labeling of the ganglion cells by the lipophilic dye 4DI-10ASP (DiA) indicated that Panx1 stains cytoplasmic organelles, plasma membranes and processes in all ganglion cells. Punctate partially co-localized labeling for both DiA and Panx1 was seen in the cell of the IPL level [36]. Therefore, in addition to the processes of the ganglion cells, some other cells might express Panx1 as well.

The presence of neuronal coupling in several cell types of mammalian retina is well established [37, 38]. In some cases, a number of connexin family members were suggested to be responsible for corresponding GJ connectivity. For example, Cx36-containing GJs between AII amacrine cells and ON-cone bipolar cells are essential for normal synaptic transmission in the primary rod pathway [39, 40]. GJs mediated by Cx36 are also essential for the transmission of the signal from the rod photoreceptors to the ganglion [41], which is probably provided by the electrical synapse. Cx57 is critically important for the functioning of the coupled horizontal cells, which presumably corresponds to mediation or contribution to center-surround effects and contour enhancement [42]. The putative role of the pannexins in this sophisticated neuronal ensemble governing visual perception remains to be studied, but the abundant expression of both Panx1 and Panx2 in the retinal neurons suggests its involvement in visual signal processing and/or the development of the retinal system.

No experiments involving double-labeling with specific glial cell marker and the pannexin in the eye have been performed so far. However, a punctate pattern of expression of Panx1 which is observed in IPL layer did not particularly coincide with neuron-specific staining [36], and may suggest that this protein is present in the glia. Rodent retina possesses two types of glial cells - retinal astrocytes and Müller cells. It was suggested previously that glia may modulate neural activity in the retina [43]. The glial-neuronal communication could be mediated by intercellular glial Ca^{2+} waves that have demonstrated influence on electrical activity of neighboring neurons. These waves propagate GJ-mediated release of ATP, and can be initiated by mechanical stimulus and be attenuated by the application of the purinergic receptor antagonists in both astrocytes and Müller cells [44]. These features of the glial-neuronal interactions are reminiscent of the properties of Panx1 protein described above. Therefore, pannexins are possible candidates for regulation of glia-neuron activity modulation in retina.

It is intriguing that significant expression of Panx1 in murine eye presents initially at E14, increases at E18 and steeply declines at birth and the subsequent postnatal period remaining low through the adulthood [34]. It is peculiar that expression of the major neuronal connexin Cx36 could not be registered in embryonic and perinatal stages, as its expression in the retina starts about P5 [45, 46]. These findings underscore the differential temporal patterns of expression for the pannexin and connexin encoding genes, and suggest a possible role of pannexins in retinal development.

In addition to retina, the expression of the Panx1 has been found in the primary non-immortalized human lens epithelial cells. Expression profiling experiments revealed that in these cells PANX1 mRNA levels significantly increase four hours after dexametasone treatment [47]. Posterior subcapsular cataract due to the perturbation of lens epithelial cell proliferation and differentiation is a well

known side effect of prolonged corticosteroid therapy. Precise mechanism of cataractogenesis is unknown, but it does involve GJ formations [48, 49]. It is of interest that exposure to dexametasone does not lead to significant changes in the levels of mRNA synthesized by any connexin encoding gene [47], thus leaving an interesting possibility for the involvement of the pannexins in cataract formation.

Expression of Panx1 and Panx2 in olfactory bulb

Investigation of the expression of pannexins in the olfactory bulb (OB) revealed abundant expression of Panx1 and Panx2 in a number of layers. Ray *et al.* (2005) found that in the OB of adult mice Panx1-specific labeling is prominent both in external tufted neurons and the mitral cell layer, while in the granule cell layer expression of this gene was faint to absent [34]. Expression of both pannexins was reported in rat OB in pups (P1, P7, P15), young animals (P30) and adults [12, 50]. In adult rats, the strongest signal for both pannexins was registered in mitral cell layer and also in accessory OB. In the granule cell layer of rat OB, Panx1- and Panx2-specific probes stained almost all cells, however, a higher extent of the staining could be seen in some unidentified scattered cells [50]. Our own experiments of in situ hybridization of Panx2 mRNA to adult mouse brain slices revealed significant labeling in the mitral cell layer as well as scattered labeled cells in external plexiform, glomerular and granule cell layers [Fig.1 A, E].

Presence of GJs in the OB is described in literature [60] and may be important functionally. Odors elicit both β-frequency (15–40 Hz) and γ-frequency (40–75 Hz) oscillations in the mammalian olfactory bulb [51–53]. These oscillations may reflect odor quality coding [54], but also have been linked to memory [55] and experience-dependent phenomena such as expectancy [56]. The presence of dendrodendritic GJs in the external plexiform layer of the olfactory bulb has long been known [57]. GJ-mediated coupling was also shown in granule layer, where GJs provide significant, low-resistance electrical transmission between aggregated granule cells [58]. Mitral cells with dendrites in the same glomerulus are probably coupled, whereas mitral cells projecting to different glomeruli are never coupled [59]. Recent electron microscopic data revealed that GJs in mitral/tufted cells are connecting their dendrites to the dendrites of similar cells, periglomerular cell dendrites and some interneurons different from periglomerular cells [60]. These findings are in marked contrast with observations of neuronal GJs in the hippocampus and the cerebral cortex where specific types of inhibitory interneurons are electrically coupled almost exclusively to interneurons of the same type [61].

It is of interest that the labeling for the main neuronal connexin Cx36 is most intense and widespread in the mitral cell layer and recognizable in the glomerular layer [46]. It also could be observed in scattered cells in the external plexiform layer (periglomerular cells and tufted cells) and in the granular cell layer [46]. In Cx36 knock-out mice electrical coupling as well as correlated spiking between mitral cells projecting to the same glomerulus is entirely absent [62]. However, the authors directly verified that Cx36 is responsible for GJs only between distal dendrites of mitral cells in olfactory bulb glomeruli. GJs in other cells in the olfactory bulb were not investigated in detail [62]. In addition, the staining for the connexins Cx32 [63], Cx43 [64] and Cx45 [65] is also found in the olfactory bulb, but their functional significance in electrical coupling in OB is thus far unknown. Given the demonstrated presence of pannexins in different cell types in OB, we expect that future investigations could shed light on their role in the processing of olfactory information.

Expression of pannexins in cerebellum

Early studies of the pannexin expression in the cerebellum of young rats revealed abundant Panx1 positive cells in its white matter of the cerebellum [12]. Staining of Panx2 was absent in the white matter, but was highly visible in Purkinje cell layer [12]. Experiments with mouse brain confirmed Panx2-specific labeling in Purkinje cells [6]. Detailed *in situ* hybridizations performed by Ray et al. on murine brain demonstrated intense staining for Panx1 mRNA in the Purkinje cells, deep cerebellar nuclei and scattered large cells in the granule layer that probably correspond to Golgi or Lugaro neurons [34]. The double labeling in principal neurons of cerebellum revealed that all Panx1-positive Purkinje cells were calbindin-

(which serves as marker for Purkinje cells in the cerebellum) and parvalbumin- (a marker for GABAergic cells) immunoreactive. At the same time, the Panx1 mRNA expressing neurons in the granule cell layer were entirely devoid of parvalbumin and calbindin immunoreactivity [34]. The cells in the molecular layer were found to be Panx1-negative [34]. Observations of pannexin expression in the rat cerebellum [50] mainly coincided with findings in mouse specimens (Table 2). Some cells in granular cell layer, possibly Golgi cells were found to contain large amounts of mRNAs for both genes. Since Panx1 expression was found both in internal and external granule cell layers of the cerebellum at P7, it was assumed that Panx1 might be expressed in proliferating neurons. Double-labeling experiments with Panx1 and proliferation marker Ki67 indeed revealed that Panx1 could be expressed both in proliferating and non-proliferating neurons [50]. Vogt *et al.* (2005) proposed non-gap-junction function of Panx1 in cells, found in the external granule cell layer, since these cells are migrating and unlikely to be coupled [50].

Our own data describing the Panx2 distribution in mouse brain coincide for the most part with Panx1 expression patterns described above (Fig.1. B, F) and feature significant expression in Purkinje cells and in scattered cells in the granular layer, and faint expression in the molecular layer. In addition, we observe faint but distinct labeling for Panx2 probe in the white matter of the cerebellum (Fig.1.F, black arrows). Interestingly, some Panx1-specific staining could be seen in the white matter of the rat cerebellum on the slides prepared by other researchers [12, 50]. It is worthwhile to note that the thorough examination of *in situ* hybridization slides representing other sites of white matter localization, *e.g.* corpus callosum (Fig.3 G) and fornix (Fig.3, B) did not demonstrate any Panx2-specific labeling. This indicates that if pannexins are indeed expressed in glial cells, their expression could be location-specific. Recently, study of Zappala *et al.*, 2006 revealed expression of Panx1 in Bergmann glial cells of the cerebellum, where it was co-localized with GFAP antibody [120]. As Panx1 expression is already proven for Purkinje cells, these findings make Panx1 an attractive candidate for recently found GJs between Bergmann glia and Purkinje cells [121]. Bergmann-Purkinje GJs are possibly involved in the development and the maintenance of cerebellar cortical network [122]. At the same time, no other glial cells investigated in this study were shown to be Panx1-positive [120].

In the rat cerebellum the expression of Panx1 is clearly detectable at P1, P7, P15 and P30, then its levels become somewhat decreased with age [50]. In mice cerebella, Ray *et al.* (2005) also showed developmental regulation of Panx1 [34]. RT-PCR experiments indicated that Panx1 is found in the cerebellum already at E16, peaked at E18, and decreased at P0 towards P14 [34]. Panx2 is characterized by inverse temporal expression pattern: in rats its mRNA levels increase from younger ages to P30, and then drop to a moderate but steady level in adult [50].

GJ-type coupling between cells in the cerebellum was shown in several studies. Already more then 30 years ago, an existence of the electrotonic coupling was revealed in the molecular layer of the cerebellar cortex [123]. Later, by paired recordings inhibitory interneurons in the molecular layer of the cerebellar cortex were shown to be electrically coupled and capable of the generating of synchronous activity. In another study, a high incidence of dye-coupling was demonstrated using intracellular staining with biocytin [124]. Also, both electrical and dye coupling were described between Bergmann glial cells [125]. Cx36 is expressed in cerebellar molecular and granular layer, as well as in cells of deep cerebellar nuclei [46, 67], however principal cells of cerebellum, Purkinje cells, are devoid of Cx36 expression (Table 2). Pannexins are expressed in majority of cell types of the cerebellum, and are plausible candidates for the basic coordinators of the cell-cell communication in this brain area.

Expression of Panx1 and Panx2 in a hippocampus

Both pannexin-encoding genes are expressed in the stratum pyramidalis (SP) of the hippocampus and in individual neurons in the stratum oriens (SO) and stratum radiatum (SR) of 15 days old rats [12]. Specific staining for Panx2 was revealed in pyramidal cell layer in CA1, CA2 and CA3 of the adult mouse hippocampus and also in the dentate gyrus (DG), in rare cells of strati oriens and radiatum, and in both granular and polymorph layers of dentate gyrus [6, 34 and Fig. 1C, 1G]. All hippocampal Panx1-positive cells were confirmed as neurons after double-labeling with NeuN, marker of neu-

Table 2 Expression of Panx1, Panx2 and Cx36 mRNA in the CNS of adult rodents

	Panx1	Panx2	Cx36
Olfactory bulb			
Mitral cells	++[34], ++[50]	++[this paper], ++[50]	++[67]
Periglomerular and external plexiform layer	++[34],	+[this paper]	++[67]
Glomerular layer	-[34]	-[this paper]	+[67], -[46]
Granule cell layer	+/-[34], ++[50]	++[this paper], ++[50]	+[67]
Accessory olfactory bulb	+[50]	+[50]	n/a
Retina			
Ganglion cell layer	++[34], ++[36]	++[36]	++[67], ++[46]
Inner nuclear layer (amacrine cells)	++[34], ++[36]	++[36]	++[67], ++[46]
Outer nuclear level	++[34]		-[46]
Inner plexiform layer	+[34]		-[46]
Outer plexiform layer (horizontal cells)	++[36]	++[36]	-[46]
Hippocampus			
Pyramidal cells (PC) of the entire CA region	++[34],++[50]	++[2, this paper], ++[50]	+[67] PC of CA3 region, but not CA1; +[46] PC of CA3 region, but not CA1
Granule cells of dentate gyrus	++[34], ++[50]	++[2, this paper], ++[50]	-[67], -[46]
Scattered cells within stratum radiatum and oriens, at the border of granular cell layer and in the hilus of the dentate gyrus	++[34], ++[50]	++[2, this paper], ++[50]	++[67], ++[46]
Entorhinal cortex	++[50]	++[50], +[A.T., O.L., unpublished observation]	+[67]
Neocortex			
Different layers of neocortex (scattered cells).	+[34], +[50]	++[2,this paper], +[50]	+[67], +[46]
Basal telencephalon			
Piriform cortex	++[34]	++[this paper]	+[67]
Lateral globus pallidus	++[34]	+[this paper]	+[67]
Nucleus caudate putamen (scattered cells)	++[34]	++[this paper]	+[67], +[46]
Nucleus accumbens	+[34]	++[this paper]	+[67]
Medial septum	+[34]	+[this paper]	+[67]
Lateral septum	+[34]	+[this paper]	n/a
Diagonal band of Broca	+[34]	+ [this paper]	+[67]
Ventral pallidum	+[34]	+ [this paper]	n/a
Bed nucleus of stria terminalis	+[34]	+ [this paper]	+[67]
Olfactory tubercle	+[34]	+[A.T., O.L., unpublished observations]	n/a

Table 2 Expression of Panx1, Panx2 and Cx36 mRNA in the CNS of adult rodents

	Panx1	Panx2	Cx36
Amygdala, dorsolateral nucleus	+[34]	+ [this paper]	-[67], -[46]
Amygdala, basolateral nucleus	+[34]	+ [this paper]	-[67], -[46]
Amygdala, basomedial nucleus	-/+[34]	n/a	-[67], -[46]
Thalamus			
Geniculate nuclei of thalamus	+[34]	n/a	-[67]
Medial and lateral habenular nuclei	+[34]	+[this paper]	+[67], +[46]
Zona incerta	+[34]	+[this paper]	+[67]
Thalamic reticular formation	++[34]	+[this paper]	++[67]
Posterior thalamus	+[34]	+[this paper]	-[67], -[46]
Lateral posterior and posterior nucleus	+[34]	+[this paper]	-[67]
Hypothalamus			
Magnocellular neurons of the paraventricular hypothalamic and supraoptic nucleus	+[34]	+[this paper]	+[67]
Arcuate nucleus	+[34]	++[this paper]	+[67]
Ventromedial hypothalamic nucleus	+[34]	++[this paper]	+[67]
Lateral division of mamillary region	+[34]	+[this paper]	+[67]
Medial subnuclei of mamillary region	-[34]	+[this paper]	+[67]
Ventral tegmental/supramamillary area	+[34]	+[this paper]	n/a
Midbrain			
Midbrain superior colliculus (large cells in deep layers)	+[34]	n/a	+[67]
Midbrain inferior colliculus (outer and inner nuclei)	+[34]	n/a	+[67]
Periaqueductal grey matter	+/-[34] (weak to absent)	+/- (rare scattered cells) [A.T., O.L., unpublished observation]	+[67]
Substantia nigra pars compacta and reticulata	++[34]	++[2]	+[67]
Hindbrain			
Medial vestibular, cohlear and cuneate nucleus	++[34]	+[this paper]	+[67]
Nucleus facialis	n/a	+[this paper]	+[67]
Pontine nuclei	n/a	+[this paper]	+[67]
Mesencephalic trigemical nucleus	-[34]	+[this paper]	n/a
Inferior olive	+[34]	n/a	++[67], ++[46]
Cerebellum			
Purkinje cells	++[34], ++[50]	++[6, this paper], ++[4]	-[67], -[46]
Molecular layer	-[34],	+[this paper]	+[67], +[46]
Granule cell layer	-[34]	-[this paper]	+[67], +[46]
Scattered large somata suggestive Golgi or Lugano neurons	++[34], ++[50]	+[this paper]	+[67] (suggested)

Table 2 Expression of Panx1, Panx2 and Cx36 mRNA in the CNS of adult rodents

	Panx1	Panx2	Cx36
Deep cerebellar nuclei	+[34]	+[this paper]	+[67]
White matter of cerebellum	++[12], +[50]	+[this paper], -[12]	n/a
Bergmann glial cells of the cerebellar cortex	+[120]	n/a	n/a
Fiber systems (white matter)			
Corpus callosum	+[50], +/-[66], -[34]	-[this paper]	-[67], -[46]
Fornix	+[50]	-[this paper]	-[67], -[46]
Spinal cord	+[12], +[34]	++[12]	+[67]

++ abundant staining; + some staining; +/- faint to absent staining; - no staining; n/a - not assessed

ronal somata. Some Panx1-positive neurons were SMI-immunoreactive (served as a marker for subsets of hippocampal pyramidal neurons) and some – parvalbumin-immunoreactive (marker for GABAergic cells of hippocampus). However, there was no co-localization of Panx1 with GFAP in the hippocampus, which suggests absence of Panx1 expression in glial cells in this brain region [34].

Vogt *et al.* (2005) found that expression of Panx1 in rat brain gradually increases postnatally resulting in a peak expression around P7 that is later decreases with age [50]. Levels of Panx2 in prenatal brain were low, its peak expression was achieved on postnatal day 15, and also was characterized by some decrease in adults. In rat hippocampus expression of both pannexins was present in DG and all CA regions. This expression was not restricted to the pyramidal cell layer but was also observed in cells located in the stratum oriens, radiatum and lacunosum-moleculare [50]. Both excitatory and inhibitory cells of the hippocampus express the two pannexin genes [50]. Weickert and co-authors recently showed that Panx1 level of expression is superior to that of Cx36, Cx47 and Cx45 in the hippocampus of adult mice [66].

All aforementioned rodent data on expression of pannexins in hippocampus are in good agreement with each other. It is of interest that the patterns of Panx1 expression in adult rodents are in marked contrast with patterns of Cx36, which is neither expressed in stratum granulosum of DG nor in pyramidal cells in CA1, but only in some principal cells of CA3 region and in GABA interneurons located in the various layers of CA1, CA3 and DG [67]. This difference between pannexin and Cx36 expression might have important functional implication. Cx36 knockout mice are able to produce ultra-fast, GJs dependent oscillations in pyramidal cell layer of CA1 and CA3 hippocampal regions [68, 69]. On the other hand, cellular synchronization during sharp wave-ripple complexes, especially its high-frequency component requires electrical coupling [70]. This finding suggests that some other GJ forming molecules are contributing to these oscillations. Whether these molecules are the pannexins is a matter of future investigations. Their localization in principal neurons of hippocampus is an encouraging factor, since high frequency oscillations are thought to be dependent on axo-axonal coupling of principal cells [71, 72]. High frequency network oscillations ("ripples") are suggested as carrier of coordination during specific information transfer between neocortical and hippocampal cell ensembles during memory consolidation [73, 74]. If pannexins are confirmed as the structural correlate for generation of ripple activity in hippocampal network new avenues for studies of memory consolidation may be opened.

Furthermore, there are direct indications that Panx1 and Panx2 could participate in hippocampal plasticity under some types of stimulation. It has been shown recently that the expression of Panx1 and Panx2 is regulated in an activity dependent manner in slices of the rat hippocampus after tetanic stimulation, which modulates the electrical

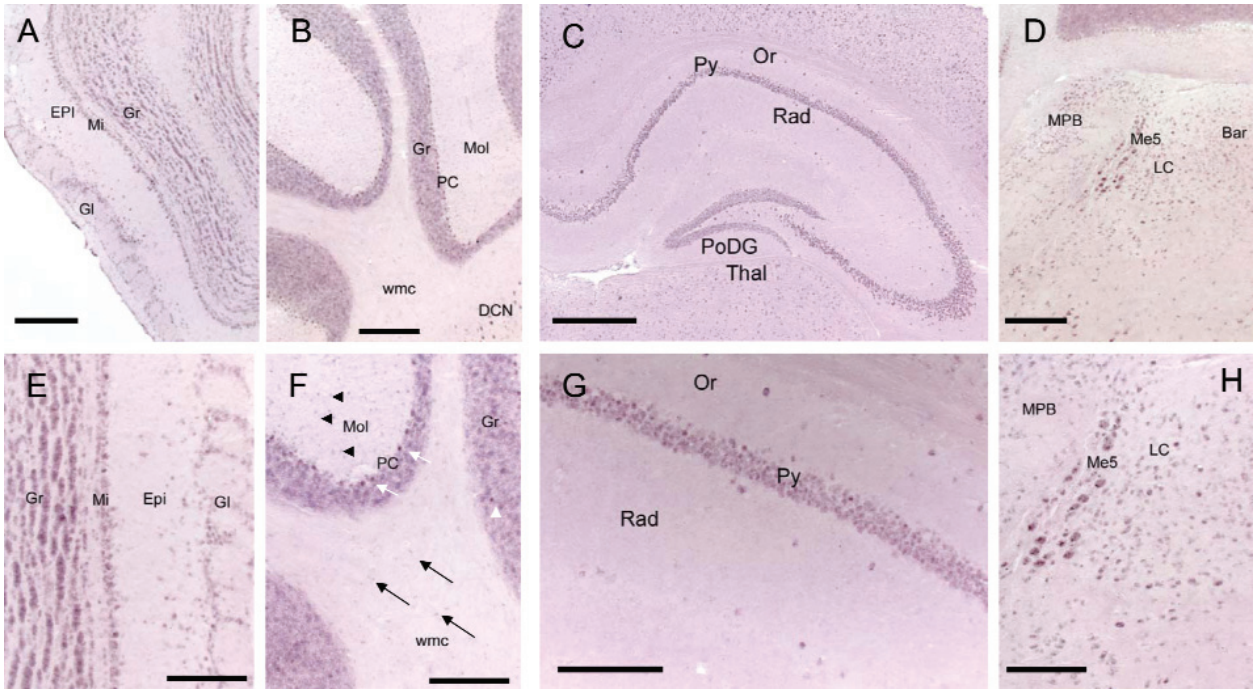

Fig. 1 Expression of Panx2 in the brain of adult mice. **A**, **E** - olfactory bulb (Gr - granular layer, Mi - mitral cell layer, Gl - glomerular layer, EPl - external plexiform layer); **B**, **F** - cerebellum (PC - Purkinje cells layer, Gr - granular cells layer, Mol -molecular cell layer, DCN - deep cerebellar nuclei, wmc - white matter of cerebellum, white arrows point at expression in PC, white arrowhead - on expression in Gr, black arrows - on expression in wmc, black arrowheads - on expression in Mol); **C** - Hippocampus, **G** - area CA1 of hippocampus (Py - pyramidal cells layer, Or - stratum oriens, Rad - stratum radiatum, PoDG - polymorph layer of the dentate gyrus, Thal - thalamus); **D**, **H** - Locus coeruleus (LC) (MPB - medial parabrachial nucleus, Me5 - mesencephalic trigeminal nucleus, Bar - Barrington's nucleus). Scale bars: **A**, **B**, **D** - 300 μm; **E**, **F**, **G**, **H** - 200 μm; **C** - 500 μm.

coupling strength [35]. Five-fold decrease of Panx1 expression in CA1, and 3-fold increase of Panx2 mRNA levels in CA3 were observed [35]. It is of interest that while six different hippocampal connexins were tested in the same experimental conditions (Cx26, Cx32, Cx36, Cx43, Cx45, Cx47), only the expression of Cx32 was changed, increasing 2-fold in the DG, and decreasing 2-fold in CA1. It is peculiar that Cx32 is not expressed in neurons, but in oligodendrocytes [75–77]. The connexins that are likely to be expressed in neurons (*i.e.* Cx36, Cx45) were not regulated in this stimulation paradigm [35].

After 2.5 hrs induction of epileptiform activity using 4-aminopyridine (an agent inducing GJ-dependent seizures) Panx2 levels were increased 1.5 fold selectively in DG. After 6 hrs of stimulation, Panx1 expression was changed significantly, increasing 1.6 fold in the DG and decreasing 5-fold in CA1 [35]. These findings are consistent with the hypothesis that pannexins are involved in electrical coupling of principal neurons since the changes in the levels of corresponding mRNAs may lead to a variation of the composition of Panx1/Panx2 channels and may modulate their coupling strength [35]. However, another recent study which exploited 4-aminopyridine-induced seizures in mice as a model of stimulation of GJ expression in hippocampus, revealed about 1.4-fold decrease in Cx36 expression 4 hours after seizures, and 3 to 5-fold decrease 8 hours after seizures by both RT-PCR and Western blot, while no change in Panx1 expression was found at any time point tested [120]. The difference in the results of these two studies could be explained by using the different animal model also differing in age: 3–4 weeks old rat *vs.* adult mice, and different stimulation paradigm: application of 4-aminopyridine to hippocampal slices to induce epileptiform activity *vs.* induction of seizures in

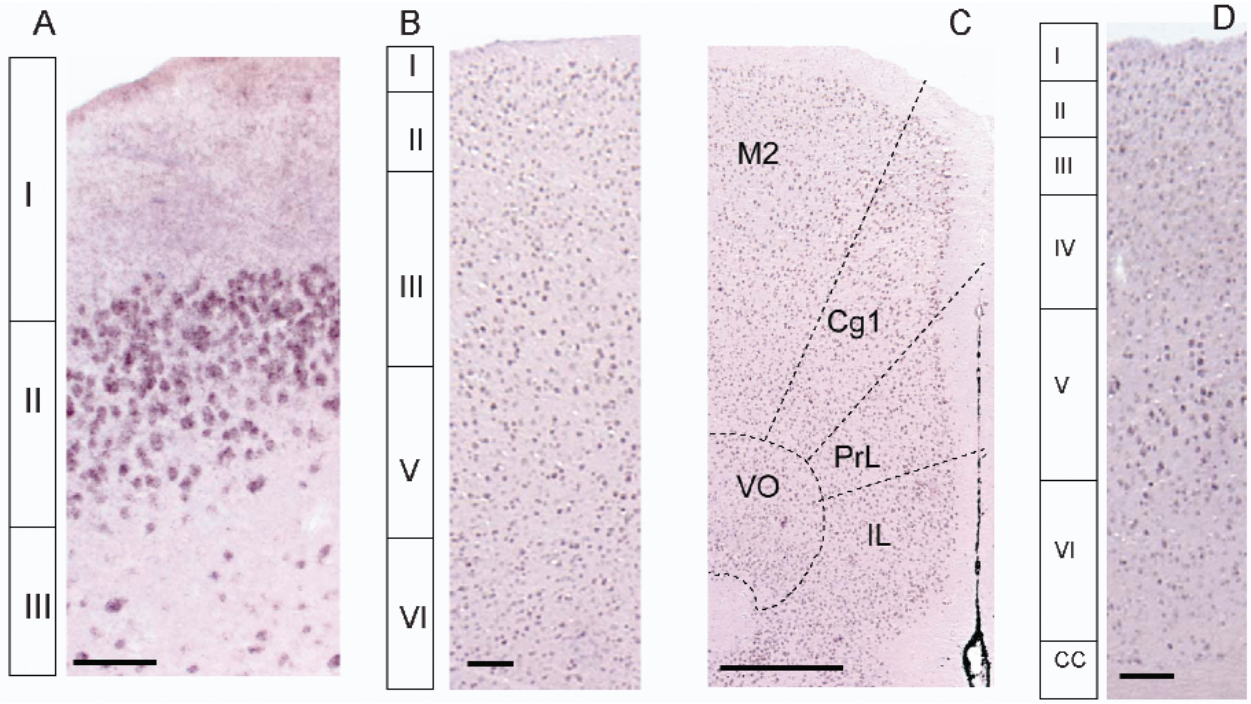

Fig. 2 Expression of Panx2 in cerebral cortex of adult mice. **A** - Piriform cortex, **B** - Primary motor cortex, M1, **C** - Prefronttal cortex (M2 - secondary motor cortex; Cg1 - cingulate cortex, area 1; Prl - prelimbic cortex, IL - infralimbic cortex; VO - ventral orbital cortex); **D** - Primary somatosensory cortex, S1. (Roman numbers denote cortical layers). Scale bars: **A**, **B**, **D** - 100 µm, **C** - 500 µm.

live animals via intraperitoneal injection. Thus, further investigations are needed to define crucial factors affecting epileptiform-dependent regulation of pannexins in the hippocampus.

Expression of the pannexins in a cerebral cortex

Both Panx1 and Panx2 are prominently expressed in the cortex of young rats (P15) [12]. In cerebral cortex of adult rats the highest levels of Panx1 and Panx2 expression were observed in the prefrontal and entorhinal regions. Cells in layers II/III and V exhibit a stronger signal for pannexin mRNAs compared to cells in layers I, IV and VI [50]. Co-immunostaining with NeuN and GFAP confirmed that expression of pannexins is exclusive to neuronal cells [50]. While approximately 95% of the parvalbumin-containing GABAergic cells in the cerebral cortex also expressed Panx1 and Panx2, the percentage for calretinin-positive neurons expressing Panx1 or Panx2 was much lower [50]. In the neocortex of adult mice expression of Panx1 was observed in cells scattered throughout different layers, with the highest staining intensity in infragranular layers (V and VI) [34]. *In situ* hybridization of Panx1 mRNA was combined with immunohistochemistry to different neuronal markers to characterize the neuronal phenotypes. All Panx1-expressing cells in murine cortex were confirmed as neurons by NeuN immunoreactivity, the same as for the rat cortex. Double staining with pyramidal cells marker SMI indicated that some Panx1 expressing cells were pyramidal cells; other Panx1-expressing cells were non-pyramidal as they co-express parvalbumin, calbindin or calretinin, markers of interneurons [34]. Our own data of expression of Panx2 in adult mouse cerebral cortex indicated that its expression varies depending on the type of cortex and layer. Highest expression was found in the piriform cortex (Fig. 2A), with most intensive labeling in layer II, and in prefrontal cortex (Fig. 2C), where all layers were evenly and intensively labeled by Panx2. In the primary motor cortex the expression was most abundant in layer III, with

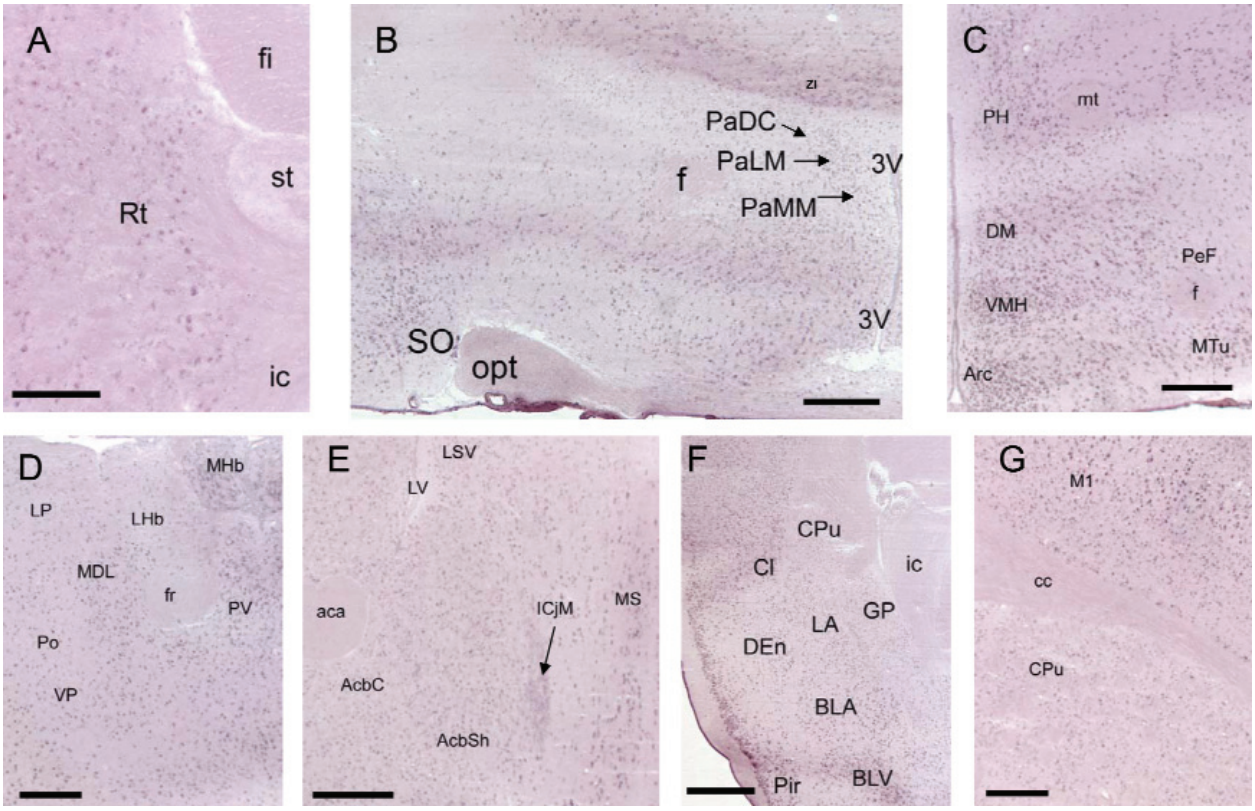

Fig. 3 Expression of Panx2 in the brain of adult mice. **A**, **D** - thalamus (Rt - reticular thalamic nucleus, fi - fimbria of the hippocampus, st - stria terminalis, ic -internal capsule, fr - fasciculus retroflexus, LHb - lateral habenular nucleus, MHb - medial habenular nucleus, LP - lateral posterior thalamic nucleus, MDL - mediodorsal thalamic nucleus, lateral part, Po - posterior thalamic nuclear group, PV - paraventricular thalamic nucleus, VP - ventral posterior thalamic nucleus); **B**, **C** - hypothalamus (ZI - zona inserta, PaDC - paraventricular hypothalamic nucleus, dorsal cap, PaLM - paraventricular hypothalamic nucleus, lateral magnocellular part, PaMM - paraventricular hypothalamic nucleus, medial magnocellular part, 3V - 3rd ventricle, SO - supraoptic nucleus, opt - optic tract, f - fornix, mt - mammillothalamic tract, Arc - arcuate hypothalamic nucleus, DM - dorsomedial hypothalamic nucleus, PH - posterior hypothalamic area, PeF - perifornical nucleus, MTu - medial tuberal nucleus, VMH - ventromedial hypothalamic nucleus); **E** - Nucleus accumbens and major island of Calleja (aca - anterior commissure, anterior part, LV - lateral ventricle, LSV - lateral septal nucleus, ventral part, AcbC - accumbens nucleus, core, AcbSh - accumbens nucleus, shell, ICjM - islands of Calleja, major island, MS - medial septal nucleus); **F** - Amygdala (ic - internal capsule, CPu - caudate putamen, GP - globus pallidus, Pir - piriform cortex, DEn - dorsal endopiriform nucleus, LA - lateral amygdaloid nucleus, BLA - basolateral amygdaloid nucleus, anterior part, BLV - basolateral amygdaloid nucleus, ventral part), **G** - Caudate putamen (M1 - primary motor cortex, cc - corpus callosum, CPu - caudate putamen). Scale bars: **A**, **G** - 200 μm, **B**, **C**, **D**, **E** - 300 μm, **F** - 500 μm.

some significant expression in layers V and VI (Fig. 2B). In the primary somatosensory cortex expression was most noticeable in layer V (Fig. 2D). In addition, neurons in the superficial and deep layers of the primary auditory cortex were found to express large quantities of mRNA for Panx1 and Panx2 [78]. Like in other brain areas, Panx1 expression in cerebral cortex peaks at E18 and is maintained at low level in adulthood [34]. Therefore, developmental patterns of pannexin expression differ from those of Cx36 that peak for embryonic stage at E15 for brain lysates [79] and peak at P12 for postnatal period in neocortex of rats [46].

In the cerebral cortex, neuronal synchronization is essential for various processes including sensory perception, motor control, attention and plasticity [80–82]. The mechanisms governing cortical synchrony are unknown. Most likely, synchrony is main-

tained by electric and chemical synapses between GABAergic interneurons [83]. Given the localization of both pannexins in GABAergic neurons of cerebral cortex, it seems plausible that they may participate in the processes of neuronal synchrony.

Expression of pannexins in other brain structures

Expression of pannexins is detected across numerous brain structures. Some of these structures are known to have electrical coupling, while in other brain areas it has not yet been investigated. Among the structures having known by both electrical synapses and demonstrated pannexin expression are the inferior olivary nucleus [34, 66] and the reticular thalamus [34]. In the former Panx1 mRNA co-localizes with calbindin, a marker of olivary neurons, and in the latter with parvalbumin [34]. Our own experiments also revealed significant expression of Panx2 in scattered cells in thalamic reticular nucleus of adult mice (Fig. 3A). A number of other thalamic nuclei also express Panx1 and Panx2 [34; Fig. 3D; Table 2).

The locus coeruleus is a small cluster of widely projecting noradrenergic brain-stem neurons possessing electrical synapses that participate in synchronization of subthreshold rhythms [84, 85] and is implicated in the modulation of arousal and attention [86]. Fig.1 (D, H) shows that scattered cells in the locus coeruleus are positive for Panx2 mRNA.

In hypothalamus, GJs may function in the secretion of oxytocin and vasopressin [87, 88]. Intensity of dye-coupling and the number of dendrodendritic membrane contacts between neuroendocrine cells in supraoptic nucleus increases in response to dehydration, gestation and lactation. Both Panx1 [34] and Panx2 (Fig. 3B) are expressed in the supraoptic and paraventricular nuclei of adult mouse hypothalamus. One of the high-throughput gene expression studies indicated that pannexins may be involved in the process of neurosecretion, as Panx1 gene was downregulated in all three independently obtained stable neurosecretion incompetent clones of PC12 cells [89]. In addition, both pannexins are expressed in number of other murine hypothalamic nuclei (34; Fig. 3C; Table 2).

Some evidence points toward the presence of the GJs between medium spiny neurons of striatum [90, 91] and at electrical coupling between its local inhibitory neurons [92]. Panx1 mRNA was found in scattered neurons of the striatum, intensely labeled, while spiny stellates cells were negative [34]. Our experiments indicated expression of Panx2 mRNA in some unidentified cells of caudate putamen, perhaps of striatal interneurons (Fig. 3G). Panx2 expression is also noticeable in the nucleus accumbens (Fig. 3E), but the major island of Calleja was devoid of labeling (Fig. 3E), which is in agreement with data of other researchers [34]. Interestingly, in spite of the absence of pannexins in cells of islands of Calleja, these cells are known to be electrically coupled [126]. Finally, noticeable expression of Panx1 [34] and Panx2 (Fig. 3F; Table 2) was found in amygdala.

Conclusions that could be made from observation of pannexins expression in rodent brain

Panx1 and Panx2 mRNAs are widely present in the brain structures of adult rodents, though their expression profiles are far from ubiquitous. By multiple research groups, Panx1 and Panx2 expression registered both in areas of brain where neurons known as electrically coupled and in the areas where electrical coupling was not yet described. Some known electrically coupled neurons are devoid of pannexins staining. Speaking generally, patterns of the pannexin expression resemble those of the main neuronal connexin, Cx36. Hovewer, differences are striking in the principal cells of hippocampus and cerebellum, where Panx1 and Panx2 are abundantly expressed, while Cx36 is absent. Investigation of the cellular localization of Panx1 protein in the principal cells of hippocampus and cerebellum revealed puncta on the cellular membrane, obviously suggestive of putative GJs [120]. Among suggested roles of pannexins in nervous systems are sensory processing, hippocampal plasticity, synchronization between hippocampus and cortex, neurosecretion and the coordination of the calcium waves supported by glial cells.

Pannexins and cancer

It is well known that GJs are involved in tissue homeostasis and that they regulate and control cell proliferation, differentiation, and apoptosis, although the mechanistic aspects of these actions remain largely unknown

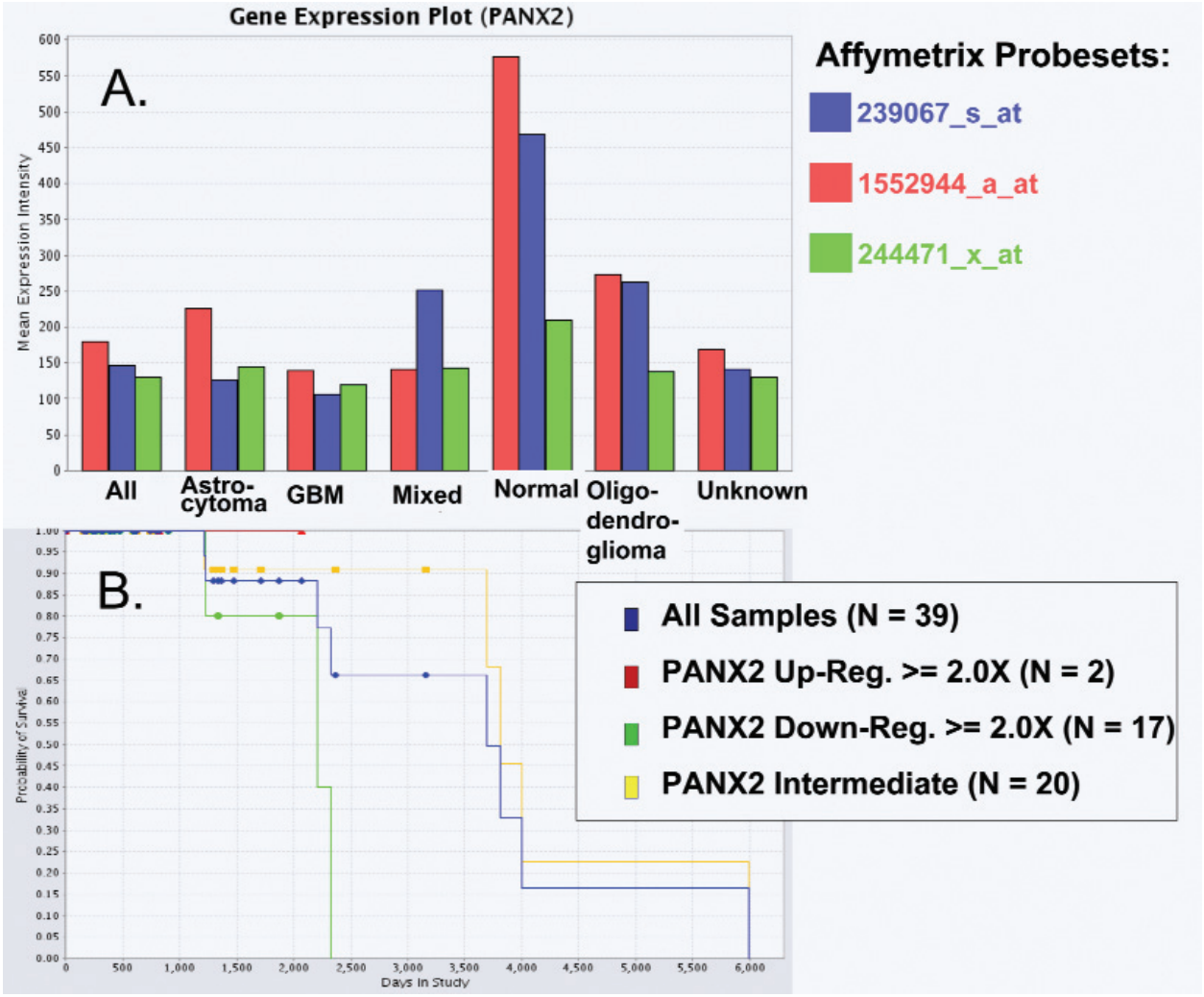

Fig. 4 Expression of the PANX2 mRNA in a human brain tumor samples. **A**. Gene expression plot that displays average expression intensities for the for PANX2 gene based on Affymetrix GeneChip arrays (U133 Plus 2.0 arrays) expression in human brain tumor samples. **B**. Kaplan-Meier Survival Plot for Samples with Differential PANX2 Gene Expression measured by unified probeset reporter 56666. Down-Regulated *vs.* Intermediate: $P < 0.023$; Down-Regulated *vs.* all other samples, $P < 0.0194$; Intermediate *vs.* all other samples, $P < 0.0325$. The log rank p-values are calculated using Mantel-Haenszel procedure.

[93]. Disruption or alteration of such communications may lead to aberrant cell growth and tumor development [94]. Connexin-encoding genes could serve as tumor suppressors [95, 96]. For example, transformed cells transduced with connexin genes have been shown to regain proliferation control, thereby suggesting that connexins do in fact have tumor-suppressor activity [97]. The most conclusive evidence of connexin depletion in tumorigenesis was recently collected for gliomas and glioblastomas [95, 98]. However, GJs have also been shown to stimulate the invasion of malignant gliomas [99, 100]. This picture became even more complex after the discovery of pannexins. As it was already mentioned in chapter about pannexin's properties, they exhibit a remarkable sensitivity to blockade by CBX, a classical inhibitor of GJ communication [13]. It is quite possible that CBX-inhibited cell migration of glioma cells described before [101] is due to a suppression of pannexin channels.

Evidence of pannexin involvement in the process of tumorigenesis is provided by high-throughput studies revealing differential expression of pannexin encoding genes in tumor samples and in model systems. A search for pannexin encoding genes in the brain cancer gene

expression database REMBRANDT (Repository of Molecular Brain Neoplasia Data, http://rembrandt.nci.nih.gov/rembrandt) yields statistically significant PANX2-based predictions of post diagnosis survival for patients with glial tumors (Fig. 4). These findings are further strengthened by the observation that the human PANX2 gene is located within chromosomal region 22q13.3 which is often deleted in human astrocytomas and ependymomas [102–105]. Thus, PANX2 could be viewed as a candidate tumor suppressor gene involved in gliomagenesis. On the top of that, in one recent study a Panx2 proteolitic fragment present into the bloodstream was identified as a potential biomarker representing a part of the serum signature of ovarian carcinoma [106]. Despite the obvious suggestion that the release of Panx2 fragments into the bloodstream of ovarian cancer patients may simply accompany the paraneoplastic process in some non-malignant tissues, it is tempting to speculate that an underlying loss of GJs is relevant to at least some manifestations of this malignancy.

Evidences of PANX1 and PANX2 involvement in tumorigenesis are even less convincing. Nevertheless, we will review them here, as they still might point us to an interesting facet of pannexin polyfunctionality. High-throughput microarray analysis of the mouse hepatocarcinoma cell line Hca-F with a metastasis rate over 70% and its syngeneic cell line Hca-P with a metastasis rate less than 30% in order, revealed that Panx1 over-expression is characteristic for metastatic cancer spread [107]. PANX1 gene amplification leading to its overexpression has been found in 2 out of 5 multiple myeloma cell lines studied, corroborating the hypothesis that this gene can serve as an oncogene [119]. However, some other studies contradict to this point of view. The amount of PANX1 mRNA greatly increases after the inhibition of the low-grade serous carcinoma MPSC1 cell proliferation as measured by LongSAGE procedure [108]. The MPSC1 cell line possesses a BRAF mutation that leads to constitutive activation (phosphorylation) of its downstream target, mitogen-activated protein kinase (MAPK), also known as extracellular signal regulated protein kinase (ERK). This activation could be prevented by treatment with a highly potent and selective inhibitor of MEK1/2, CI-1040 (PD184352) leading to the profound suppression of the proliferation. Pronounced over-expression of Panx1 after CI-1040 treatment was confirmed by Real-Time PCR both in MPSC1 cell line and in two other ovarian carcinoma cell lines that contain activating KRAS mutations [108]. The only observation linking Panx3 to the tumorigenesis is an identification of this protein as a molecular partner of BCL6, a transcription factor involved in lymphomagenesis [110]. This finding came from co-immunoprecipitation experiments with an anti-BCL6 antibody and subsequent tandem MS/MS spectroscopy [109].

At this point, it is difficult to say whether pannexins can be viewed as tumor suppressors or oncogenes unequivocally. Most likely, pannexins represent yet another group of the genes whose expression is disturbed in tumors. Similarly to connexins, particular pannexins may restrain initial growth of the tumor in situ, but hasten the metastatic spread of the existing malignancy. Future studies of the pannexin connection to cancer are warranted.

Conclusion

The ability to form the intercellular channels in paired oocytes and hemichannels in single oocytes has been demonstrated for rodent pannexins. Hypothetical role of pannexins in nervous systems exploiting both channel and hemichannel forms involved participating in sensory processing, hippocampal plasticity, synchronization between hippocampus and cortex, and propagation of the calcium waves supported by glial cells, which help maintain and modulate neuronal metabolism. Pannexin also may participate in pathological reactions of the neural cells: there is evidence, that ischemia opens neuronal GJ hemichannels, which properties resembles those of pannexins, and that leads to neuronal death. Recent study revealed non-GJ function of Panx1 hemichannels in erythrocytes, where it functions as conduit for release of ATP in response to osmotic stress. Beside that, there are indications that pannexins may also be involved in tumorigenesis particularly of gliomagenesis.

Acknowledgements

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